Stress-Induced Colibacillosis and Turkey Osteomyelitis Complex in Turkeys Selected for Increased Body Weight

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ABSTRACT Two stress models were used to induce colibacillosis and turkey osteomyelitis complex (TOC): *Escherichia coli* challenge following dexamethasone injection (Dex) and *E. coli* challenge preceding transport stress (Transport). A total of 160 birds from 3 lines of turkeys: a slow-growing line selected for egg production (Egg), a line selected for 16-wk BW (F line), and a Commercial line (Comm), were studied in a $3 \times 3 \times 2$ (line × treatment × sex) factorial design. At 14 wk, the Dex group was treated with 3 injections of 2 mg of Dex/kg of BW followed by airsac challenge with 100 cfu of *E. coli*. The Transport group was given 5,000 cfu of the same *E. coli* and 8 d later was transported for 3 h and held for an additional 9 h in the transport vehicle. Controls of each line were neither stressed nor challenged with *E. coli*. Birds were necropsied 2 wk postchallenge. All birds were sexed, scored for airsacculitis (AS) and TOC, and knee synovia were cultured for *E. coli*. Percent mortality was unaffected by sex, was increased by the Dex treatment, and was higher in Dex-treated male Comm-line birds and Dex-treated female F-line birds compared with their respective nonchallenged controls. Both treatments increased AS scores, and scores of Dex-treated male Comm-line birds and female F-line birds were also higher compared with their respective controls. Male Comm birds under Transport had higher AS scores as compared with nonchallenged males and challenged females. The TOC incidence was increased by Dex only. There was no TOC in Egg-line birds, whereas TOC incidence approached significance in both Comm and F lines compared with the Egg line ($P = 0.06$). Males had twice as much TOC as females, and this approached significance in the F line ($P = 0.06$). There was a low level of TOC in male Transport birds of both large-bodied lines, whereas no female Transport birds had TOC lesions. Dex-treated male birds of both the F line and Comm line had significantly higher incidence of TOC compared with their respective nonchallenged controls. The challenge strain of *E. coli* was isolated from more knee cultures of both large lines compared with the Egg line. Isolation was increased by Dex and was higher in male Comm-line birds and both male and female F-line birds relative to their controls. The difference in disease resistance between these lines suggests that selection for fast growth of turkeys may affect the stress response, resulting in increased chronic bacterial disease such as TOC.

Key words: turkey, transport stress, dexamethasone, body weight, *Escherichia coli*

2006 Poultry Science 85:266–272

INTRODUCTION

The genetic selection of poultry for superior growth rate has been responsible for much of the increased productivity of the modern poultry industries; however, many studies have shown that such selection may be coincidentally accompanied by decreased resistance to disease or changes in immunological response (Han and Smyth, 1972; Saif et al., 1984; Saif and Nestor, 2002; Sacco et al., 1991, 1994a,b, 2000; Tsai et al., 1992; Miller et al., 1992; Qureshi and Havenstein, 1994; Nestor et al., 1996a,b,c, 1999a,b; Li et al., 1999, 2000a,b,c, 2001; Cheema et al., 2003). It has been suggested that genetic variation in the stress response greatly complicates the development of poultry lines with high levels of immunocompetence (Gross and Siegel, 1988; Siegel, 1995).

Recently, Kowalski et al. (2002) compared 2 lines of commercial European turkeys, a faster growing heavy line and a slower growing medium line from the same breeder, for their physiological responses to the stressors of transport, crowding, and overheating. They reported that the faster growing line was more sensitive to adverse environmental factors and had a much larger increase in corticosterone when exposed to transport stress than the slower growing line. They suggested that lighter and slower growing lines may be more suitable for certain

Received August 25, 2005.
Accepted October 21, 2005.
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commercial production situations due to their adaptability to stress.

Unapparent, chronic infection of joints, bones, and soft tissues with opportunistic bacterial pathogens is a problem in commercial turkey production. This condition, referred to as turkey osteomyelitis complex (**TOC**), causes significant condemnation of carcasses since the USDA Food Safety and Inspection Service mandated that processed turkeys be inspected for these lesions (Cook, 1988). An experimental model has been developed that consistently reproduces all of the lesions of TOC using dexamethasone (**Dex**) treatment followed by airsac challenge with low levels (50 to 100 cfu) of *Escherichia coli*, *Staphylococcus aureus*, or *Arcanobacter pyogenes* (Huff et al., 1998, 1999). This research model supports the hypothesis that TOC is caused by the effects of production stress on a susceptible subpopulation of male turkeys (Huff et al., 1999, 2000).

The effects of 2 different stress models, transport stress and Dex treatment, on changes in the stress response as measured by heterophil/lymphocyte (**H/L**) ratio in 3 genetic lines of turkeys that differ in their rate of growth has recently been reported (Huff et al., 2005). The purpose of the present study was to describe the effects of these 2 stress models on the incidence of TOC and the presence of bacteria in the knee synovial tissues of challenged turkeys.

MATERIALS AND METHODS

Three genetic lines of turkeys were compared for their responses to stress and *E. coli* challenge. The turkey lines were a slow-growing line selected exclusively for increased egg production over a 250 d period (**Egg**), another line selected for increased 16-wk BW (**F**), and a Commercial line (**Comm**). The birds from the Egg and F line were the progeny of a hatch of eggs obtained from the Ohio Agricultural Research and Development Center, Wooster, Ohio. The hatch consisted of 66 Egg-line birds and 42 F-line birds of mixed sex. Fifty Comm poults of mixed sex were obtained from a commercial turkey hatchery at d of age and were set in pens on the same day as the closed lines. All turkeys were reared in floor pens on pine shavings, given ad libitum access to a standard corn and soybean turkey ration meeting or exceeding the NRC recommended allowances (National Research Council, 1994), and were kept under incandescent lighting on a light schedule consisting of 23 h day and 1 h night. For the first 2 wk the birds were brooded under heat lamps in a single pen for each line. At 2 wk of age they were separated into 18 pens in a 3 line × 3 treatment design with 2 replicate pens for each group. Five or 6 birds were placed into each of the control pens, and 7 to 10 birds were placed into each of the challenge pens where they were maintained until 13 wk of age. All research involving animals was evaluated and approved by the Institutional Animal Care and Use Committee of the University of Arkansas.

Dexamethasone and E. coli Challenge

At 13 wk of age, 1 group was immunosuppressed with 3 injections of the synthetic glucocorticoid, Dex (Sigma Chemical Co., St. Louis, MO) into a thigh muscle at a dosage of approximately 2 mg of Dex/kg of BW as previously described (Huff et al., 1998). A 200 mg/mL stock solution of Dex was prepared in absolute ethanol. This solution was suspended in sterile normal saline and an average volume of 1.0 mL was inoculated into each bird, based on a mean body weight of 2.73 kg for the Egg line, and 6.49 kg for the 2 large-bodied lines. Birds were weighed the day before the first Dex injection, and the amount each bird received was determined using a running scale correlating body weight and volume. On the day of the third Dex injection (14 wk of age), all Dex-treated birds were inoculated in the left cranial-thoracic air-sac with sterile tryptose phosphate broth containing approximately 100 to 200 cfu of a nonmotile strain of *E. coli* serotype O2, which had originally been isolated from chickens with colisepticemia. The inoculum was prepared by adding 2 inoculating loops of an overnight culture on blood agar to 100 mL of tryptose phosphate broth and incubating for 2.5 h in a 37°C shaking water bath. The culture was held overnight at 4°C while a standard plate count was made. Ten-fold dilutions were then made in TPB based on the standard plate count.

Transport Stress

Birds in the transport stress treatment were similarly challenged with approximately 5,000 to 10,000 cfu of the same *E. coli* culture. A 50-fold higher level of bacteria was used for transported birds as compared with Dex-treated birds because the effects of environmental stressors on individual birds is highly variable, unlike the more general immunosupression resulting from glucocorticoid treatment. These birds were subjected to the following transport stress procedure, which occurred 8 d after the bacterial challenge and included a total of 12 h of holding time in the transport vehicle: Birds were loaded into an open-fenced trailer covered with a tarpaulin and with the Egg line separated from the F and Comm lines by a fence to protect them from the F and Comm lines. The temperature ranged from 18 to 21°C, and there was a slight drizzle. The birds were driven around the University farm facilities for 3 h with occasional stops. They were then driven to the University Pilot Processing Plant, where the transport vehicle was parked in a covered holding area. After a total of 12 h from time of loading, birds were returned to their original pens, provided with feed and water, and necropsied the following morning.

Necropsy

Mortalities were collected twice each day after challenge and were weighed, sexed, and examined for lesions of airsacculitis (**AS**) and turkey osteomyelitis complex. The following key, modified from that described by

Table 1. Effect of 2 stress models, transport stress and dexamethasone (Dex) injection, on percentage mortality of male and female 15-wk-old turkeys from 3 genetic lines[1]

Treatment	Egg line		F line		Comm		Main effect mean (treatment) $P = 0.001$
	Male	Female	Male	Female	Male	Female	
	(%)						
Control	0 ± 0	0 ± 0	14 ± 14	0 ± 0[b]	0 ± 0[b]	0 ± 0	3[b]
Transport[2]	0 ± 0	10 ± 10	33 ± 21	0 ± 0[b]	22 ± 15[b]	22 ± 15	16[b]
Dex[3]	12 ± 12	17 ± 17	25 ± 25	50 ± 22[a]	60 ± 16[a]	37 ± 18	36[a]
Main effect mean (line, $P = 0.07$)	8		22		27		
Main effect mean (sex, $P = 0.54$)							
Male	21						
Female	18						

[a,b]Means within a row or column with no common superscript differ significantly ($P \leq 0.05$).

[1]A slow-growing line selected exclusively for increased egg production over a 250-d period (Egg line), a line selected for increased 16-wk BW (F line), and a commercial line (Comm).

[2]Transport stress consisted of injection of approximately 5,000 to 10,000 cfu of *E. coli* into the airsac, 8 d before a 12 h transport and holding procedure.

[3]The Dex treatment consisted of 3 i.m. injections of 2 mg of Dex/kg of BW on alternating days followed by airsac injection of 100 to 200 cfu of *E. coli* on the day of the last Dex injection.

Piercy and West (1976), was used to score lesions of AS and pericarditis observed in both mortalities and at necropsy: 0 = no inflammation; 1 = opacity and thickening of the inoculated air sac; 2 = mild AS and mild pericarditis; 3 = moderate AS/pericarditis with spread to liver or abdominal cavity (perihepatitis/peritonitis); 4 = severe fibrinous AS and severe pericarditis; and 5 = severe AS/pericarditis with spread to liver and/or abdominal cavity. Incidence of TOC was determined using the 10-cut procedure of the Food Safety and Inspection Service (Cook, 1988). Liver, airsac, knee synovial tissue, and all TOC lesions were swabbed with sterile transport swabs (Bacti-Swabs, Remel, Lenexa, KS). At 15 wk and 4 d of age, all surviving birds were weighed and necropsied as described previously.

For both mortalities and necropsied birds, transport swabs were immediately taken to the laboratory where they were plated on MacConkey agar, mannitol salt agar, and Columbia blood agar (Remel). Representative lactose-negative colonies on MacConkey agar were identified using API 20-E test kits according to the manufacturer's instructions (BioMérieux Vitek Inc, Hazelwood, MO) and were compared with the challenge strain. Recovered isolates were further characterized and compared with the challenge strain using the BioLog Microbial ID system (Biolog, Inc., Hayward, CA).

Statistics

Pen means were analyzed as a $3 \times 3 \times 2$ factorial arrangement (line × treatment × sex) using the GLM procedure of SAS software and means were separated using Duncan's multiple range test (SAS Institute, 1988). Differences between each treatment relative to untreated controls and between lines and sex within treatments were separated using the least square means procedure of SAS software. A *P*-value of less than 0.05 was considered significant unless otherwise stated.

RESULTS

The main effect mean for percentage mortality was significantly increased by the Dex treatment compared

Table 2. Effect of 2 stress models, transport stress and dexamethasone (Dex) injection, on airsacculitis scores of male and female 15-wk-old turkeys from 3 genetic lines[1]

Treatment	Egg line		F line		Comm		Main effect mean (treatment) $P < 0.0001$
	Male	Female	Male	Female	Male	Female	
Control	0 ± 0	0 ± 0	0 ± 0	0 ± 0[b]	0 ± 0[b]	0 ± 0[b]	0.0[c]
Transport[2]	0.6 ± 0.6	1.1 ± 0.5	0.6 ± 0.6	0.7 ± 0.5[b]	2.3 ± 0.6[a]	0.8 ± 0.6[ab]	1.1[b]
Dex[3]	1.2 ± 0.7	0.8 ± 0.8	1.7 ± 0.6	3.2 ± 1[a]	2.8 ± 0.7[a]	2.1 ± 0.8[a]	2.0[a]
Main effect mean (line, $P = 0.1$)	0.76		1.1		1.5		
Main effect mean (sex, $P = 0.5$)							
Male	1.17						
Female	1.12						

[a-c]Means within a column with no common superscript differ significantly ($P \leq 0.05$).

[1]A slow-growing line selected exclusively for increased egg production over a 250-d period (Egg line), a line selected for increased 16-wk BW (F line), and a commercial line (Comm).

[2]Transport stress consisted of injection of approximately 5,000 to 10,000 cfu of *E. coli* into the airsac, 8 d before a 12 h transport and holding procedure.

[3]The Dex treatment consisted of 3 i.m. injections of 2 mg of Dex/kg of BW on alternating days followed by airsac injection of 100 to 200 cfu of *E. coli* on the day of the last Dex injection.

Table 3. Effect of 2 stress models, transport stress and dexamethasone (Dex) injection, on incidence of turkey osteomyelitis complex (TOC) in male and female 15-wk-old turkeys from 3 genetic lines[1]

Treatment	Egg line		F line		Comm		Main effect mean (treatment) $P = 0.005$
	Male	Female	Male	Female	Male	Female	
	(%)						
Control	0 ± 0	0 ± 0	0 ± 0[b]	0 ± 0	0 ± 0[b]	0 ± 0	0[b]
Transport[2]	0 ± 0	0 ± 0	20 ± 20[ab]	0 ± 0	11 ± 11[ab]	0 ± 0	5[b]
Dex[3]	0 ± 0	0 ± 0	50 ± 29[a]	17 ± 17	30 ± 15[a]	25 ± 16	19[a]
Main effect mean (line, $P = 0.06$)	0		13		13		
Main effect mean (sex, $P = 0.1$)							
Male	12						
Female	5						

[a,b]Means within a row or column with no common superscript differ significantly ($P \leq 0.05$).

[1]A slow-growing line selected exclusively for increased egg production over a 250-d period (Egg line), a line selected for increased 16-wk BW (F Line), and a commercial line (Comm).

[2]Transport stress consisted of injection of approximately 5,000 to 10,000 cfu of *E. coli* into the airsac, 8 d before a 12 h transport and holding procedure.

[3]The Dex treatment consisted of 3 i.m. injections of 2 mg of Dex/kg of BW on alternating days followed by airsac injection of 100 to 200 cfu of *E. coli* on the day of the last Dex injection.

with the control (Table 1). The main effect mean for line approached significance in both of the large-bodied lines as compared with the Egg line ($P = 0.07$). There was no difference in the main effect mean for sex, and there were no significant interactions for mortality. Comparing means within lines, the Dex treatment increased mortality of male Comm turkeys and female F-line turkeys. There was no significant increase in percentage mortality within the Egg line by either Transport or Dex treatment relative to untreated controls.

Main effect mean AS scores were increased by both Transport and Dex treatment (Table 2). There was no effect of line or sex on AS scores, and there were no significant interactions. Comparing means within lines, the Dex treatment resulted in significantly higher AS scores in female F-line birds and female Comm birds. Both Transport and Dex treatment increased AS scores in male Comm birds. Neither treatment affected AS scores of the Egg-line birds.

The main effect mean incidence of TOC was increased by Dex treatment (Table 3). The main effect mean for line approached significance at the level of $P = 0.06$. Males had twice the level of TOC incidence of female turkeys ($P = 0.1$), and this effect approached significance in the F line ($P = 0.06$). There was a low level of TOC in males of both large-bodied lines that underwent Transport stress, whereas no females had TOC lesions. There were no significant interactions for TOC incidence. Comparing means within lines, there was no TOC seen in Egg-line birds regardless of treatment, and TOC incidence was significantly higher in male F-line and Comm-line birds in the Dex treatment compared with untreated male controls of their respective lines.

In most cases, knee synovial tissue swabs from challenged birds yielded pure cultures of the challenge strain of *E. coli*. The challenge strain of *E. coli* was isolated from the knee synovial tissues of significantly more F-line and Comm birds than from Egg-line birds (Table 4). The main effect mean for Dex treatment was significantly higher as compared with the control. The main effect mean for sex was not significant, and there were no significant interactions.

Table 4. Effect of 2 stress models, transport stress and dexamethasone (Dex) injection, on percent isolation of challenge strain of *Escherichia coli* from knee synovial tissue of male and female 15-wk-old turkeys from 3 genetic lines[1]

Treatment	Egg line		F line		Comm		Main effect mean (treatment) $P = 0.001$
	Male	Female	Male	Female	Male	Female	
	(%)						
Control	0 ± 0	0 ± 0	0 ± 0[b]	0 ± 0[b]	0 ± 0[b]	0 ± 0	0[b]
Transport[2]	0 ± 0	10 ± 10	33 ± 21[ab]	0 ± 0[b]	22 ± 15[b]	11 ± 11	13[b]
Dex[3]	0 ± 0	17 ± 17	50 ± 29[a]	67 ± 21[a]	67 ± 17[a]	25 ± 16	38[a]
Main effect mean (line, $P = 0.03$)	5[b]		25[a]		23[a]		
Main effect mean (sex, $P = 0.3$)							
Male	23						
Female	20						

[a,b]Means within a row or column with no common superscript differ significantly ($P \leq 0.05$).

[1]A slow-growing line selected exclusively for increased egg production over a 250-d period (Egg line), a line selected for increased 16-wk BW (F line), and a commercial line (Comm).

[2]Transport stress consisted of injection of approximately 5,000 to 10,000 cfu of *E. coli* into the airsac, 8 d before a 12 h transport and holding procedure.

[3]The Dex treatment consisted of 3 i.m. injections of 2 mg of Dex/kg of BW on alternating days followed by airsac injection of 100 to 200 cfu of *E. coli* on the day of the last Dex injection.

Comparing means within lines, bacterial isolation was higher in male Comm birds and both male and female F-line birds compared with their respective controls.

DISCUSSION

These data suggest that intense selection for performance may compromise immunity by altering the birds' response to stress and support the conclusions of Kowalski et al. (2002), who suggested that lighter commercial turkey lines might be more suitable for commercial poultry production than heavier lines because of their better response to stress. The increase in TOC incidence and joint colonization with *E. coli* also implies that faster growing birds may harbor opportunistic pathogens within their tissues to a greater extent than slow-growing birds, suggesting an opportunity for increasing food safety by improvement of the stress response of commercial turkeys or by decreasing the overall production stressors to which commercial turkeys are exposed.

There are many varied effects of stress on the immune system, but it has been suggested that the basic mechanism of Dex immunosupression involves interference with cytokine synthesis due to transcriptional activation of the I κ B α protein (Scheinman et al., 1995). Although this mechanism can affect the function of many cells, one of the major side effects of either glucocorticoid treatment (Anderson et al., 1973; Gustafson et al., 1983) or endogenous hypercortisolism (Graham and Tucker, 1984) is an increased susceptibility to opportunistic infections that may be related to the ability of glucocorticoids to interfere with the bactericidal ability of macrophages (Schaffner, 1985; Schaffner and Schaffner, 1988).

The ability of Dex to prevent *Listeria*, *Nocardia*, and *Salmonella* killing by mammalian macrophages, without impairing their ability to phagocytose these organisms (Schaffner, 1985; Schaffner and Schaffner, 1988) suggests that the stresses involved in commercial turkey production could have a similar effect in reducing bactericidal efficiency. These latent infections may then recrudesce in joints, synoviae, and growth plates when the natural life span of the cell ends and bacteria are released. Infection would be more likely to occur in stressed or otherwise immunosuppressed birds.

It is well known that although moderate levels of stress can be immunosuppressive, they sometimes serve to improve resistance to certain types of infection (Siegel, 1980; Gross et al., 1980). It is also clear that although steroid treatment is known to impair immune response and decrease macrophage bactericidal activity, it also results in more heterophils in birds (Siegel, 1968) and neutrophils in human patients (Yamamoto and Friedman, 1996). Under certain experimental conditions, the effect of neutrophilia might serve to increase resistance to certain target organisms, explaining the divergent findings on the effects of steroid treatment, especially in an intravenous model. In the Dex model for turkey stress immunosupression (Huff et al., 1998, 1999, 2000, 2005), both peripheral blood heterophil and monocyte numbers are increased by Dex treatment, indicating that phagocyte function rather than number might have been responsible for the increase in infection. McGruder et al. (1995), have similarly reported that intraperitoneal injection of 5 mg of Dex/kg resulted in a 7-fold increase in heterophil numbers in the peripheral blood of chickens but did not increase resistance to experimental *Salmonella* infection, suggesting that these heterophils may not be fully functional. It is interesting that the superior resistance seen in the Egg line in the present study was accompanied by significantly lower percentages of both heterophils and monocytes in peripheral blood compared with the F line and Comm line (Huff et al., 2005), suggesting that these cell populations might be less efficient at bacterial killing in the large-bodied turkey lines. The H/L ratio, a widely used measurement of stress in birds (Gross and Siegel, 1983) was lower in the egg line than in the 2 fast-growing lines, and although both stress treatments increased the H/L ratio, the effect was significantly greater in both fast-growing lines compared with the Egg line (Huff et al., 2005).

The immunosuppressive effects of stress may be greater in male birds than in females (Redig et al., 1985; Huff et al., 1999), and female turkeys are more resistant to the Dex-*E.coli* challenge than are males (Huff et al., 1999). Turkey osteomyelitis complex is a disease that primarily affects male turkeys between the ages of 9 and 20 wk that are beginning to develop secondary sexual characteristics and is not considered to be a problem in females (Nairn, 1973; Clark et al., 1991; Mutalib et al., 1996). While both males and females had increased TOC incidence in the Dex challenge, it is interesting that in the transport treatment, which is more physiologically relevant to actual turkey production, TOC was only seen in fast-growing male birds. The current data contribute to the hypothesis that the combined effects of disease, management, and environmental stressors may decrease resistance to opportunistic bacterial infection in genetically susceptible, fast-growing male turkeys.

It seems apparent that the response to stress has been altered by selection for increased BW, particularly in male turkeys, suggesting that incorporation of stress models into the selection process may be needed to further develop high performance lines that are more stress tolerant and disease resistant. It is well known that genetic variation in the responses to varied environmental and social stressors greatly complicates the development of poultry lines with high levels of immunocompetence (Gross and Siegel, 1988; Siegel, 1995). The current emphasis of society on both food safety and animal welfare has raised the expectations of consumers. Further study is needed to genetically select birds with an improved ability to tolerate the stressors inherent in commercial poultry production, and in nutritional, behavioral, and environmental strategies for modulating the stress response.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the excellent technical assistance of D. Bassi, S. Tsai, S. Zornes, D. Horlick, L. Stamps, and W. McDonner.

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